

Gestational exposure to methylmercury and *n*-3 fatty acids: Effects on high- and low-rate operant behavior in adulthood

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Abstract

Fish in the diet is the major source of methylmercury (MeHg) exposure, but eating fish also provides important nutrients. Many fish species contain essential long chain polyunsaturated fatty acids, especially docosahexaenoic acid (DHA), an omega-3 (or *n*-3) fatty acid, that is important for neural development and function. To examine interactions between MeHg and *n*-3 fatty acids, female Long-Evans rats were exposed, *in utero*, to 0, 0.5, or 5 ppm MeHg via drinking water, approximating exposures of 0, 40, and 400 µg/kg/day. They also received pre- and postnatal exposure to a diet containing either fish oil or coconut oil, creating a 2 (Diet) × 3 (MeHg) full factorial design, with 6–8 rats per cell. The diets were high or marginal, respectively, in *n*-3 fatty acids but approximately equal in *n*-6 fatty acids. No exposure-related effects on developmental milestones or growth were noted. Behavior was evaluated using a series of rapidly increasing fixed ratio (FR) schedules of sucrose reinforcement; 1, 5, 25 and 75 lever presses were required for sucrose delivery, with three sessions provided at each requirement. This phase was followed by four sessions of a differential-reinforcement-of-low-rate-behavior (DRL) schedule, in which presses preceded by 10 s (or more) without a press were reinforced. Subsequently, several progressive ratio (PR) schedules that increased response requirements throughout a single session by a rate of 5%, 10%, or 20% were imposed. Rats exposed during gestation to MeHg had significantly higher response rates than controls under the large FR schedules, during the first session of DRL, and the PR 5% schedule, but neither fish oil nor coconut oil modified MeHg's effects. This finding is consistent with hypotheses that developmental MeHg exposure produced perseverative responding or altered the sensitivity of behavior to its reinforcing consequences and that certain reinforcement contingencies can unmask MeHg's effects.

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1. Introduction

The developing brain is especially vulnerable to methylmercury (MeHg) toxicity [70], and that vulnerability has consequences that extend into adulthood and aging [33,42,47,55]. Recent human epidemiological studies have sought relationships between developmental exposure to MeHg and children's cognitive and motor development, but the results are equivocal. A longitudinal study of Seychelles Islanders revealed that tests of cognitive function were unaffected, or even improved, in children exposed to MeHg during develop-

ment [14,15,16]. On the other hand, in other cohorts from New Zealand [12] and the Faroe Islands [25,26], MeHg appeared to impair scores on similar neuropsychological test batteries.

The discrepancies among these studies have been attributed to any of several factors including the possibility that benefits deriving from long chain omega-3 (or *n*-3) polyunsaturated fatty acids (PUFAs) in marine fish may mask or somehow counteract MeHg's neurotoxicity [13,20,25,37]. This could account for differences between two ongoing epidemiological studies because MeHg exposure in the Seychelles population is due to a high and steady frequency of fish consumption, while exposure in the Faroe Islands population is more closely linked to the occasional but liberal consumption of marine mammals.

Essential *n*-3 PUFAs, particularly eicosapentaenoic acid (20:5*n*-3, EPA) and docosahexaenoic acid (22:6*n*-3, DHA), are

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abundant in the oil of certain fish species, and they are essential nutrients that support proper brain development including visual and motor function [20,52]. Laboratory investigations of deficiencies in various *n*-3 PUFAs have produced effects that, at least superficially, resemble those produced by prenatal MeHg exposure. Dietary *n*-3 deficiency reduces brain weight [69], impairs vision [48,53,71,72], and produces motor deficits on swim tasks [27] (also see Ref. [2]). Prenatal exposure to MeHg hinders cortical development [7,54], impairs visual function [56,57], and interferes with swimming ability [21,64]. However, upon closer inspection, the pattern of effects sometimes looks different, and different mechanisms may underlie their effects [52]. For example, DHA contributes to the formation of rhodopsin [32] while MeHg exposure damages small neurons in the retina and impairs contrast sensitivity [55].

Studies of PUFAs are not easily comparable to those investigating MeHg because the two literatures have used somewhat divergent procedures to tap learning and memory processes (see Refs. [2,41] for a review of PUFAs and MeHg, respectively). The simultaneous manipulation of MeHg and dietary PUFAs in a single laboratory investigation of behavior will foster a better understanding of how these two dietary variables interact.

Analyses of operant behavior are essential in the assessment of any neurotoxicant [41]. Laboratory procedures involving changes in the consequences of behavior can be manipulated to test how quickly a subject learns to adapt to new situations [62], and if neurotoxicant exposure disrupts this process [43]. For example, it has been shown that acquisition of responding under rapidly increasing fixed ratio (FR) schedules is influenced by developmental exposure to cadmium [45], lead [11,46], TCDD [31], and ethanol [38]. Effects on behavior transitioning to a new arrangement may even occur in the absence of effects on behavior after extended exposure to an unchanging, stable environment. This outcome has been demonstrated with developmental MeHg in situations involving choices between concurrently available reinforcement schedules [46,47].

The present investigation was designed to (a) determine whether prenatal MeHg exposure, like exposure to other toxicants, disrupts performance when response requirements increase quickly, (b) test for perseveration or impulsivity by imposing a rapid transition from a high-rate to a low-rate behavior schedule and (c) evaluate the hypothesis that *n*-3 PUFAs present in fish mitigate the effects of MeHg. The experiments were conducted using a 2 (Diet) × 3 (MeHg) full factorial design, an approach that permits the direct examination of interaction between MeHg and *n*-3 PUFAs. During gestation, rats were exposed to 0, 0.5, or 5 ppm of MeHg, levels that produce brain concentrations that are relevant to human exposures [7,44]: about 0.5–9.5 ppm at birth and about 10-fold less at weaning. They were also exposed, pre- and postnatally, to a diet that was either marginal or rich in *n*-3 fatty acids but approximately equal in *n*-6 fatty acids. In the first phase of the experiment, fixed ratio (FR) schedule requirements changed, with each requirement in effect for three consecutive sessions. In the second phase, a differential-reinforcement-of-low-rate (DRL) schedule was imposed im-

mediately following the FR phase. The DRL contrasts well with the FR schedule because, unlike the FR, the DRL permits only a single response within a specified period of time. In a final phase, progressive ratio (PR) schedules were implemented. Individual PR schedules were similar to FR schedules used in the first phase but permitted continuous increases in ratio requirements within a single session.

2. Methods

2.1. Subjects

The subjects were 46 female Long-Evans rats (F₁ generation) housed in environmentally-controlled colony rooms with a 12:12 light–dark cycle (lights on at 7:00 a.m.). Subjects were bred in the laboratory, and each was randomly selected from a different litter, so the litter served as the statistical unit in all analyses. While *in utero*, they received exposure to one of three doses of methylmercury (MeHg) and a diet either high or low in *n*-3 PUFAs, forming a 2 (Diet) × 3 (MeHg) factorial design (detailed below). Table 1 shows the number of subjects selected from each of the six exposure conditions.

After weaning on postnatal day (PND) 21, the subjects were injected subcutaneously with an electronic identification chip (Biomedic Data Systems, Seaford, DE). They were housed two per cage, separated by a transparent divider diagonally placed in the cage so that feeding could be tailored to each individual rat's requirement while maintaining adequate space requirements for each rat. During adulthood, after PND 90, their food was rationed so as to maintain their body weights at 250 g; this required approximately 15 g of food per day. Those that shared a home cage received the same diet (see Exposures) so that diets were never mixed. In order to prevent excessive tooth growth, a cleaned, nylon chew “bone” was freely available in the home cage.

As pups, the subjects were evaluated for developmental milestones. This included tests of surface righting, elevation of head, gait, eye opening, onset of walking, startle reflex, and negative geotaxis. No methylmercury- or diet-related effects were detected on these tests. For 2 months prior to the present investigation, all subjects were exposed to a behavioral test

Table 1
Exposure, age when tested, and number of subjects per age per cell group

Methylmercury (MeHg) dose	Diet			
	Coconut Oil (CO)		Fish Oil (FO)	
	Age ^a	<i>n</i>	Age ^a	<i>n</i>
0 ppm	9	4 ^b	12	8
	12	4		
0.5 ppm	12	8	12	6
5 ppm	9	5 ^c	12	8
	12	3		

^a Age in months.

^b Subjects from a second round of breeding used to control for age differences within the CO/5-ppm exposure group.

^c Subjects from a second round of breeding were included to make up for failed breeding attempts in the CO/5-ppm exposure group.

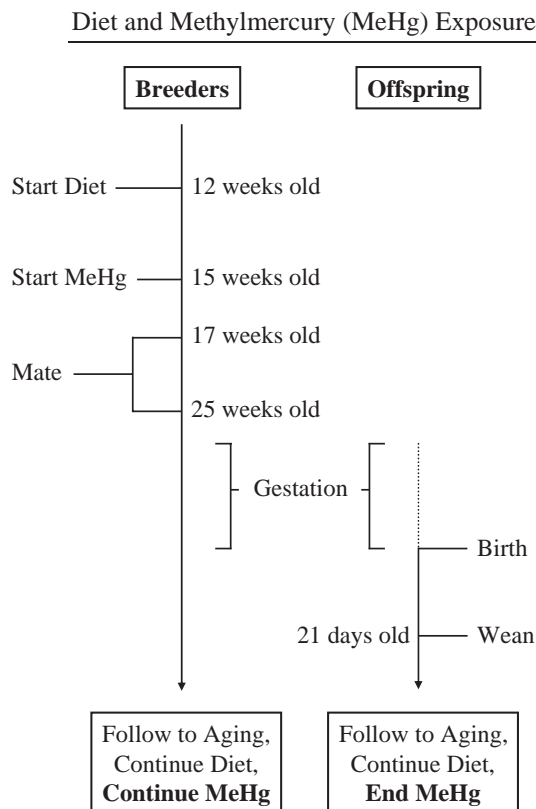


Fig. 1. Timeline for exposures and breeding for F_0 breeders, and exposures for F_1 offspring. Note exposure to methylmercury ended for offspring, including those that were used as subjects in the present experiment. Breeders were not included in the present experiment. See text for details.

involving concurrent schedules of reinforcement (as described in [47]). They were 9 or 12 months of age at the beginning of the present experiment, depending on time of breeding (see Breeding and Table 1).

2.2. Breeding

Beginning at approximately 17 weeks of age, 36 male and 72 female Long-Evans rats (F_0 generation; Harlan, Indianapolis, IN) were bred. Fig. 1 presents a timeline for exposures and breeding. Breeding cages contained the female's diet and tap water, so males were never exposed to MeHg (see Exposures). Each male was paired with a single female during every other dark cycle. Most males were paired with a second female during alternating dark cycles. A male was paired with the same female(s) throughout breeding. When a male was bred with two females, the females were always members of different exposure groups (see Exposures). Breeding of females continued until systematic increases in daily body weight were observed, suggesting gravidity. Births before 5:00 pm were assigned to PND 0 for that day. All births after 5:00 pm were assigned to PND 0 for the subsequent day. Large litters were culled and small litters combined to produce eight F_1 pups including at least three females when possible, but only one female per litter was included in the present study. Behavior of the F_0 rats will not be described here.

Due to breeding failures among F_0 females in one of the six groups (see Table 1), a second round of breeding, with new F_0 males (12) and females (24), was undertaken but only for the affected group (CO/5-ppm) and a concurrent control group (CO/0-ppm). Consequently, there were age differences at the time of behavioral testing, as indicated in Table 1. The age of females (and males), however, was the same for both breeding periods; i.e., timelines for exposures and breeding were the same (see Fig. 1).

All procedures were approved by the Auburn University Institutional Animal Care and Use Committee. The colony was housed under conditions meeting PHS guidelines, and during the course of the experiment, the facility received AAALAC accreditation. All rats were monitored daily by the research staff and personnel from the Department of Laboratory Animal Health at Auburn University.

2.3. Exposures

2.3.1. Diets

At about 12 weeks of age, 5 weeks before breeding (see Fig. 1), the F_0 females' rodent chow was replaced with a customized diet based on the AIN-93 semi-purified formulation (supplied initially by Dyets, Inc., Bethlehem, PA, and later by Research Diets, Inc., New Brunswick, NJ). The base fat mixture of the diet consisted of 42.8% palm oil, 9.2% safflower oil, and 15.0% soybean oil. For some of these rats, the fat mixture contained 33% EPAX (Pronova Biocare, Lysaker, Norway) fish oil mixture (fish oil or FO diet), making it rich in the $n-3$ PUFAs, eicosapentaenoic acid (20:5 $n-3$, EPA) and docosahexaenoic acid (22:6 $n-3$, DHA). The fat mixture for the others consisted of 33% coconut oil (coconut oil or CO diet), an oil containing no EPA or DHA. The fat mixtures contained 1.0–1.6% α -linolenic acid (18:3 $n-3$, ALA), a biosynthetic precursor to EPA and DHA, and they also contained similar concentrations of $n-6$ PUFAs, about 18% and 23% of total fats for CO and FO diets, respectively. Overall, the ratios of $n-6$ to $n-3$ PUFAs were 16.5:1 for the CO diet and 2.1:1 for the FO diet. Table 2 lists the percentages of fatty acids for each diet. A "growth" diet, consisting of 7% fat from the mixture of oils described above, was used throughout pregnancy and lactation. At all other times a "maintenance" diet, made up of 4% of the fat mixture, was used. Selenium content of

Table 2

Fatty acid content of experimental diets (% fatty acids, as weight)^a

Fatty acid breakdown	Diet	
	Coconut oil (CO)	Fish oil (FO)
Total saturated fatty acids	59.1	38.4
Total monounsaturated fatty acids	21.8	20.4
Polyunsaturated fatty acids:		
α -linolenic acid (18:3 $n-3$, ALA)	1.1	1.6
linoleic acid (18:2 $n-6$, LA)	18.1	23.2
eicosapentaenoic acid (20:5 $n-3$, EPA)	0	5.4
docosahexaenoic acid (22:6 $n-3$, DHA)	0	4.2
Total $n-3$	1.1	11.2
Total $n-6$	18.1	23.2
$n-6:n-3$ ratio	16.5	2.1

^a Mean of three analyses taken from [59].

the diet was adjusted to be 0.40 ppm during breeding and 0.18 ppm at all other times. Male breeders were maintained on rodent chow, except when briefly exposed to the female's diet during breeding (see Breeding). All F₁ offspring continued to receive the same diet as their maternal dams.

2.3.2. Methylmercury

At about 15 weeks of age, 2 weeks before breeding (see Fig. 1), the F₀ females began consuming water containing 0, 0.5, or 5 ppm of mercury as methylmercuric chloride (Alfa Aesar, Ward Hill, MA). These concentrations of MeHg produce exposures of about 0, 40 and 400 µg/kg/day, respectively, based on average daily consumption, with some elevation during gestation due to increased fluid consumption [44]. Sodium carbonate (<5 nanomolar), which can buffer the MeHg [66], was added to all three water mixtures. When the F₁ pups were capable of reaching the water spout, on PND 16, MeHg water was removed from the cage and replaced with the 0 ppm water. After weaning and throughout the remainder of life, all F₁ rats received plain tap water to drink. Male breeders received exposure to plain tap water only.

2.4. Testing apparatus

The experiments were conducted in 16 commercially purchased operant chambers (Med-Associates, Model ENV-008) containing one rear-mounted lever and two front, retractable levers (each calibrated so that 0.20 N registered a press), a pellet dispenser situated between the two front levers and filled with 20 mg sucrose pellets (Research Diets, Inc., New Brunswick, NJ), Sonalert tones™ (2900 and 4500 Hz, nominally; calibrated to 70 dBC), a house light (28 V 100 ma), and a light emitting diode (LED) above each lever. The LEDs, 2900 Hz tone and front levers, which remained retracted, were not used in the investigation. Drinking water was freely available through a custom mounted bottle with a spout to the left of the rear lever. Each chamber was surrounded by a sound-attenuating cabinet with built-in ventilating fan that circulated air into the experimental environment and provided masking white noise. Programs for experimental procedures and data collection were written using MED-PC IV (Med-Associates, Georgia, VT). Session events were recorded with 0.01" resolution.

2.5. Behavioral methods

Lever-pressing was established via autoshaping (as described by [8]). Reinforcement consisted of the delivery of one sucrose pellet paired with a brief, 4500 Hz tone. At the beginning of the study and throughout experimental testing, body weights did not differ among any of the exposure groups. Sessions for each of three squads of subjects were conducted daily at different, but consecutive, times; assignment of subjects to squads and chambers was distributed across exposure groups.

2.5.1. Fixed ratio transitions

During the first three sessions after autoshaping, a fixed ratio 1 (FR 1) schedule of reinforcement was in effect for rear

lever presses; one lever press was required for each reinforcer delivery. Subjects were then exposed to the following sequence of larger lever press requirements: FR 5, FR 25, FR 75, and FR 5, each condition lasting 3 sessions. Table 3 shows the order of conditions tested across the entire experiment, the number of sessions per condition, and the session length.

2.5.2. DRL

On the day immediately following the last FR session, the subjects were tested on a differential-reinforcement-of-low-rate 10-s (DRL 10-s) schedule, which reinforced pairs of lever presses that were separated by at least 10 s without a press. That is, to be reinforced, a response had to end an interresponse time (IRT) ≥ 10 s. If an IRT did not meet this requirement, the schedule timer was reset to zero and began incrementing again.

2.5.3. Progressive ratios

Approximately 3 weeks after the DRL phase, subjects were exposed to several progressive ratio (PR) schedules. All sessions began with FR 10 as the first ratio. After completion of the first 10 responses, the ratio requirement increased by 5%, 10%, or 20% of the previous requirement (rounded to the nearest integer), depending on the session. For example, for a session with a 5% escalation rate, the sequence of incrementing ratio requirements was 10, 11, 11, 12, 12, 13, 13, 14, 15, 16, and so on; for the 20% escalation rate, the sequence was 10, 12, 14, 17, 21, 25, 30, 36, 43, 52, and so on. Each PR escalation rate was presented twice, once in a 30-min session and once in a 150-min session.

2.6. Data and statistical analyses

All statistical analyses were performed using SPSS® 12 (SPSS Science, Chicago, IL). The Type I error rate (α) was set at 0.05 for all tests.

2.6.1. Primary analyses

A univariate repeated-measures analysis of variance (RMA-NOVA) was performed for each phase of the experiment. MeHg (0, 0.5, 5 ppm) and Diet (CO, FO) served as the two between-subjects, treatment factors, with 6–8 rats per cell (see

Table 3
Sequence of schedules presented, number of sessions, and session length

Schedule		Number of Sessions	Session Length (min.)
Fixed ratio ^a	FR 1	3	≤30
	FR 5	3	≤30
	FR 25	3	≤30
	FR 75	3	≤30
	FR 5	3	≤30
DRL 10-s ^a		4	≤30
Progressive ratios	10%	1	30
	20%	1	30
	5%	1	30
	10%	1	150
	5%	1	150
	20%	1	150

^a Sessions lasted 100 reinforcers or 30 min, whichever came first.

Table 1). Response requirement (FR), session (DRL), or escalation rate (PR) served as the repeated, within-subjects factor (WSF), depending on the phase of the experiment. Each omnibus RMANOVA permitted the detection of four within-subjects effects (WSF, MeHg*WSF, Diet*WSF, and MeHg*-Diet*WSF) and three overall, between-subjects effects (MeHg, Diet, and MeHg*Diet).

The dependent variable for each phase was response rate. Response rates for the FR were logarithmically transformed because variability was positively related to FR requirement. For the PR phase, two breakpoint measures served as additional dependent variables: the number of ratios completed per session and the largest, or maximum, ratio completed in the session. Thus, there were five planned RMANOVAs (i.e., response rates for FR, DRL and PR, as well as two additional PR breakpoint measures).

The Greenhouse-Geisser correction was always used to adjust degrees of freedom for the tests of within-subjects effects. Univariate contrasts were performed whenever there was an interaction between a treatment factor (MeHg or Diet) and the WSF in order to identify the level(s) of the WSF for which there was a difference across treatment groups. For the level(s) of the WSF for which there was a difference, post-hoc, pairwise comparisons among the three MeHg dose groups were performed to determine which differed from each other. Pairwise comparisons were unnecessary for Diet, as it involved only a single comparison. The contrasts were functionally equivalent to performing simple ANOVAs at each level of the WSF, except that the pairwise portions of the contrasts used the same mean square error term as the denominator for each F ratio.

2.6.2. Secondary analyses

For the FR phase, distributions of interresponse times (IRTs), defined as the times between consecutive responses, exclusive of times between responses with an intervening reinforcer delivery, were compiled for each subject and session. Where primary effects were found, targeted percentiles (10th, 50th, 90th) within the distribution of IRTs for each individual session were independently analyzed using simple ANOVAs (i.e., there were three separate ANOVAs per session). Whenever there was a statistically significant effect for any of these ANOVAs, post-hoc tests (Tukey HSD) were performed to compare MeHg exposure groups. For the DRL phase, response efficiency was assessed by dividing the number of reinforcers by the number of responses for each subject and session; a value of 1 reflects optimal efficiency (every IRT meets criterion and is reinforced) and a value of 0 defines a situation in which no responses are reinforced. The number of reinforcers alone were also assessed for differences among groups. For the PR phase, four new “breakpoint” measures were devised and analyzed. These were separately defined as the time into each session when the first IRT exceeded (a) 1, (b) 2, (c) 4, and (d) 8 min. RMANOVAs were used to analyze each of these secondary DRL and PR measures.

The secondary measures were examined in order to glean some understanding of what may underlie any reported effects

from the planned, primary analyses. Statistical analyses and *p* values are reported to provide a sense of the size of the difference, relative to variability, but since the comparisons were not planned the actual *p* values should be interpreted with caution.

2.6.3. Quality control measures

Several quality-control measures were undertaken routinely. Electronic identification chips were used to track subjects, and rats were scanned before and after sessions to insure they were placed in the appropriate chamber and home cage. Fans, lights, tones, levers, and pellet dispensers were tested before and after sessions for each squad of rats to ensure that equipment was functioning properly.

In order to rule out time of day as a confounding variable, raw response rates for individual sessions were compared, daily, across the three squads with a univariate ANOVA. Likewise, to determine if equipment malfunctioned, individual-session response rates were compared across the 16 operant chambers.

Since the CO/0-ppm and CO/5-ppm MeHg groups contained rats that were 9 and 12 months old (see Breeding and Table 1), comparisons between ages allowed determination of whether age was a confound. At the end of the study, univariate ANOVAs were used to compare raw response rates, averaged across all sessions of the experiment, for older rats with younger rats both within and across each of these two cell groups. All quality control ANOVAs were conducted independent of exposure-related analyses; that is, exposure was not used as a statistical factor.

3. Results

There were no significant between-subjects effects for any RMANOVA. That is, there were no main effects of either MeHg or Diet and no interaction between them when data within each of the exposure groups (see Table 1) were averaged across all FR values for the FR phase, across all sessions for the DRL phase, or across all escalation rates for the PR phase. However, treatment effects were observed at specific levels of the within-subjects factor within these phases. Therefore, within-subjects main effects and their interactions are presented below. *F* ratios, degrees of freedom and *p* values are reported for results of the omnibus tests, but only *p* values are reported for contrasts and post-hoc comparisons unless stated otherwise.

3.1. Main effects of within-subjects factors

There were significant main effects of FR response requirement [$F(4,160)=94.0$, $P<0.001$], DRL session [$F(2,80)=139.5$, $P<0.001$], and PR escalation rate [$F(2,80)=61.6$, $P<0.001$] on response rate. For DRL, there were also main effects of session on response efficiency [$F(2,80)=119.6$, $P<0.001$] and on number of reinforcers [$F(2,80)=100.4$, $P<0.001$]. For PR, there were also main effects of escalation rate on number of ratios [$F(2,80)=669.7$, $P<0.001$], on

maximum ratio [$F(2,80)=58.3, P<0.001$], and on each of the four IRT breakpoints [all F 's(2,80) $>4.9, P$'s <0.02].

3.2. Fixed ratio transitions

There was a significant interaction between MeHg and response requirement [$F(8,160)=3.3, P=0.03$]. However, there was no interaction among MeHg, Diet and response requirement [$F(8,160)=0.3, P=0.8$] or between Diet and response requirement [$F(4,160)=1.7, P=0.2$].

Fig. 2 shows that response rates for MeHg groups, irrespective of diet, increased across increasing FR values up through the FR 25. For the FO diet (top), the 5 ppm group continued to increase up through the FR 75 while the rates for the 0 and 0.5 ppm groups dropped during the transition from FR 25 to FR 75. For the CO diet (center), response rates leveled off during the FR 75 at rates similar to those during the FR 25 for both the 0.5 and 5 ppm groups while the 0 ppm

group rates dropped during the transition from FR 25 to FR 75. During the second implementation of a FR 5 schedule, response rates were slightly higher than during the initial FR 5, but they did not differ across Diet or MeHg exposures.

Since Diet did not interact with FR requirement alone or in combination with MeHg, data from the two diets were pooled in order to simplify contrasts. These contrasts indicated that the FR 75 schedule contributed significantly to the interaction between response requirement and MeHg ($P=0.05$). Fig. 2 (bottom) confirms this, showing that the 5 ppm exposure resulted in higher response rates than seen in the other two groups. Subsequent pairwise comparisons among MeHg groups indicated that the 5 ppm group rates were significantly higher than those of the 0 ppm group ($P=0.01$), but the 0.5 ppm group did not differ from the 0 ppm group ($P=0.2$); the 5 and 0.5 ppm groups did not differ either ($P=0.3$). The figure also shows that the FR 25 schedule may have contributed too, but the probability value for this contrast did not reach

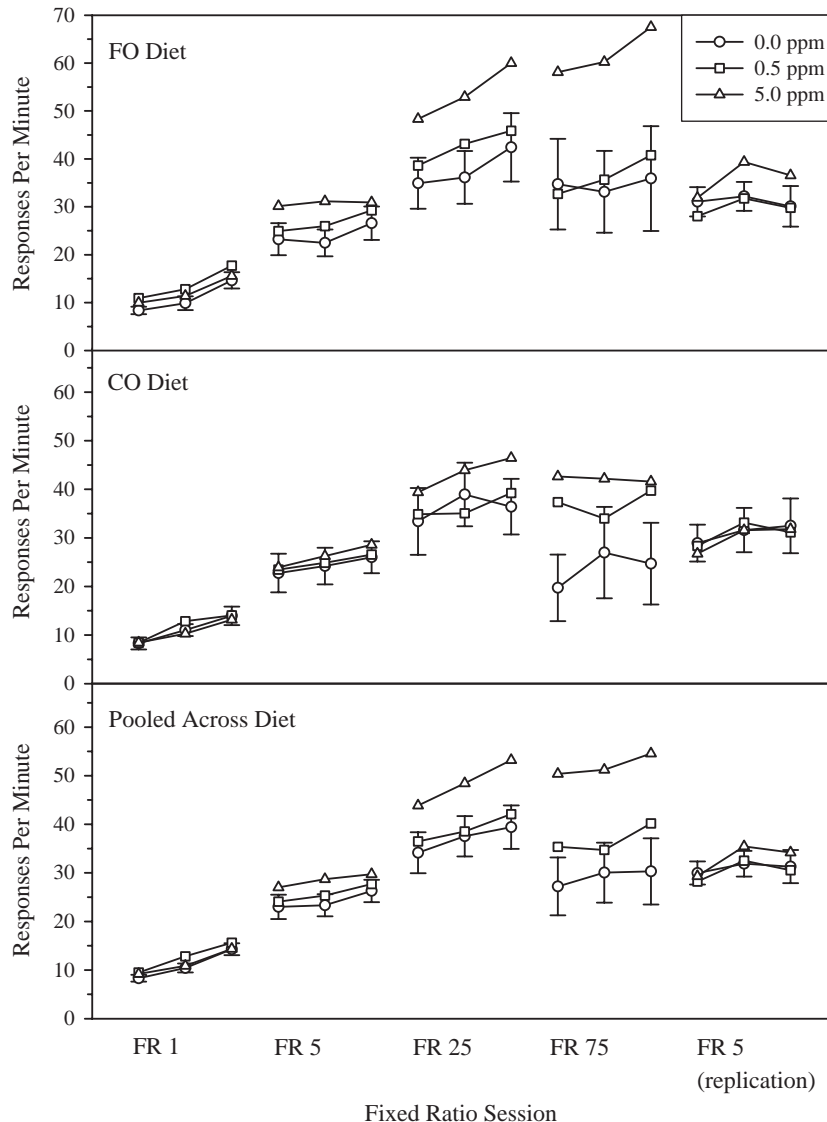


Fig. 2. Response rates across all sessions of the FR phase. Shows MeHg exposure groups for the FO diet (top), CO diet (center), and pooled across both diets (bottom). Error bars represent ± 1 SEM (for controls).

traditional levels of significance ($P=0.1$). The FR 1 and FR 5 schedules did not contribute to the interaction between FR requirement and MeHg exposure (P 's >0.2).

Visually, the plots in Fig. 2 suggest that fish-oil might interact with MeHg, especially for the FR 75 condition. Response rates for the 0.5 ppm group resembled the 0 ppm group for the FO rats but resembled the 5 ppm group for the CO rats. Inspection of individual animal data revealed why there was no significant interaction between MeHg and Diet. One rat receiving the CO/0.5-ppm exposure responded during the FR 75 sessions at rates that were roughly twice that of other rats in this treatment group. When this rat's data were removed (not shown), the graphs from the FO and CO diets groups are nearly identical.

Fig. 3 shows selected percentiles from the distribution of IRTs for the three sessions of the FR 75, separated according to MeHg dose group. During each session, median IRTs for the

5 ppm group were roughly half the duration of those from the 0 ppm group, with the median for the 0.5 ppm group generally falling between those of the other two groups, regardless of percentile. This indicates a leftward shift in the IRT distribution for the exposed rats, with the 5 ppm group showing a greater shift to the left. Statistical analyses of logarithmically-transformed means for the first session (left column) revealed that differences were marginal for the 10th [$F(2,43)=2.9$, $P=.07$], 50th [$F(2,43)=2.9$, $P=0.07$], and 90th [$F(2,43)=2.6$, $P=0.09$] percentiles, with the 5 ppm group producing marginally shorter IRTs than the 0 ppm group for all percentiles ($0.08 > P$'s > 0.05). For the second FR 75 session (center column), differences were significant for the 10th [$F(2,43)=4.4$, $P=0.02$] and 90th [$F(2,43)=3.5$, $P=0.04$] percentiles, with the 5 ppm group producing significantly shorter IRTs than the 0 ppm group for both percentiles (P 's <0.03). Similar but marginal results were obtained for the 50th

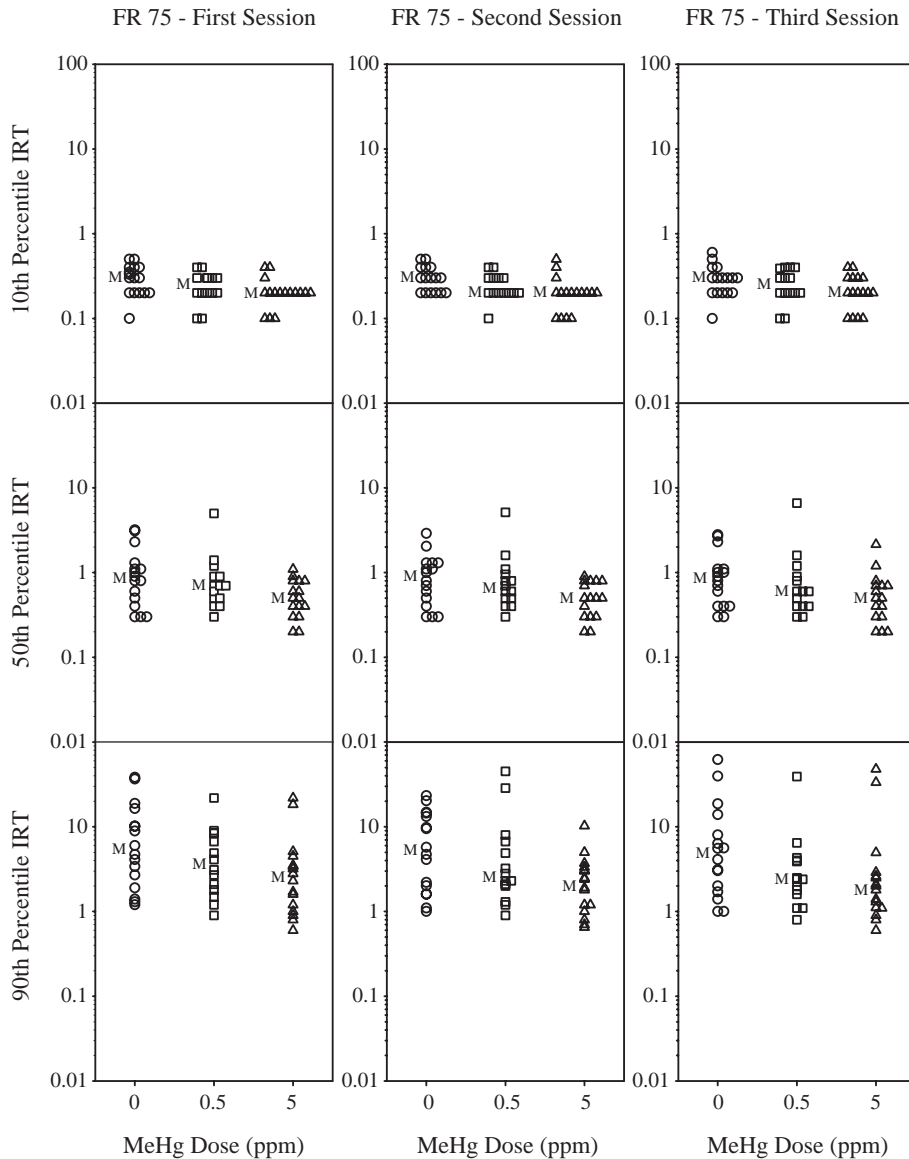


Fig. 3. Dot density plot showing interresponse times (IRTs) for the 10th percentile (top), median (center), and 90th percentile (bottom) from the first (left column), second (center column), and third (right column) sessions of the FR 75. The “M” represents the median of the group. Note log scaling on the vertical axes.

percentile IRT [$F(2,43)=3.1$, $P=0.06$]. For the third session (right column), differences among groups were not significant for any of the three percentiles [all F 's(2,43)=1.9, P 's=0.2].

3.3. DRL

Data for several subjects during the second DRL session were lost due to computer malfunction, and therefore, all data from that session were excluded from analyses.

Results revealed a significant interaction between MeHg and session [$F(4,80)=3.3$, $P=0.04$], but there was no interaction among MeHg, Diet and session [$F(4,80)=0.3$, $P=0.8$] or between Diet and session [$F(2,80)=1.5$, $P=0.2$].

Data from the diets were pooled because interactions did not involve Diet. During the first session of the DRL 10-s, response rates were about 50% higher for the 5 ppm group than for the other two groups (Fig. 4, top). By the final session, all groups were responding similarly. Initial-session response rates were highest for the 5 ppm group. Contrasts for the interaction between DRL session and MeHg indicated that response rates among the MeHg groups for the first session were significantly different ($P=0.04$), while rates among these

groups during the final session were not different ($P=0.5$). Subsequent pairwise comparisons among MeHg groups confirmed the differences observed in Fig. 4. The 5 ppm group responded significantly faster than the 0 ppm group during the first session ($P=0.03$), but there was no difference between these groups during the final session ($P=0.2$). There were no differences between the 0.5 and 0 ppm groups during either of these DRL sessions (P 's>0.4). The 5 ppm group also responded significantly faster than the 0.5 ppm group during the first session ($P=0.03$), but not during the final session ($P=0.7$).

Fig. 4 (center) also shows that response efficiency for the 5 ppm group was slightly lower than that for the other groups during the first session, although there was no interaction between MeHg and session with respect to efficiency [$F(4,80)=1.0$, $P=0.4$]. Regardless, efficiency improved across sessions for all groups, albeit it did not reach optimal levels (i.e., close to 1.0).

Despite obvious differences in response rate during the initial session, the number of reinforcers per session (Fig. 4, bottom) was roughly the same for each of the MeHg groups regardless of session, as confirmed by a lack of interaction between MeHg and session [$F(4,80)=1.5$, $P=0.2$]. The number of reinforcers increased similarly for all groups across sessions.

3.4. Progressive ratios

Although there were main effects of escalation rate for the 30-min PR sessions (not reported here), there were no effects involving MeHg, Diet or their interaction. Inspection of within-session data (i.e., cumulative records) revealed that all rats exhibited high rates of responding throughout the 30-min sessions, even at the end of sessions. Evidently, these sessions were too brief to allow a progression to ratios large enough for exposure groups to be distinguished from controls. Therefore, these data were not included in analyses and will not be discussed further.

3.4.1. Response rates

For the 150-min sessions, results revealed a significant interaction between MeHg and escalation rate [$F(4,80)=4.6$, $P=0.01$]. However, there was no interaction among MeHg, Diet and escalation rate [$F(4,80)=0.9$, $P=0.5$] or between Diet and escalation rate [$F(2,80)=0.1$, $P=0.9$].

Data were collapsed across Diet because it produced no interactions. Fig. 5 shows response rates (top) for the three escalation rates. The 0 and 0.5 ppm exposed rats responded at similar rates overall. The largest differences appeared for the 5% session, with the 5 ppm rats responding faster, on average, than the 0 or 0.5 ppm rats. Comparable differences also occurred for the 10% and 20% sessions, but these differences were less than half of those observed for the 5% session. Contrasts of individual escalation rates across all MeHg groups indicated that only the 5% rate produced a significant difference in response rate ($P=0.01$); the 10% and 20% rates did not produce a difference (P 's=0.2). Subsequent pairwise

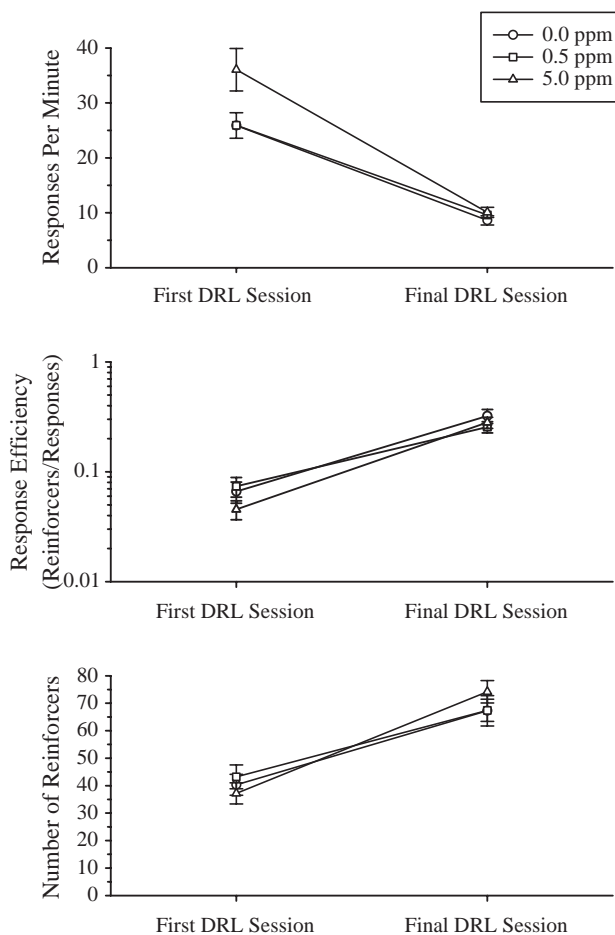


Fig. 4. Response rate (top), response efficiency (center) and number of reinforcers (bottom) for each MeHg group during the first and last DRL 10-s sessions. Note log scaling on the vertical axis of response efficiency plot. Error bars represent ± 1 SEM.

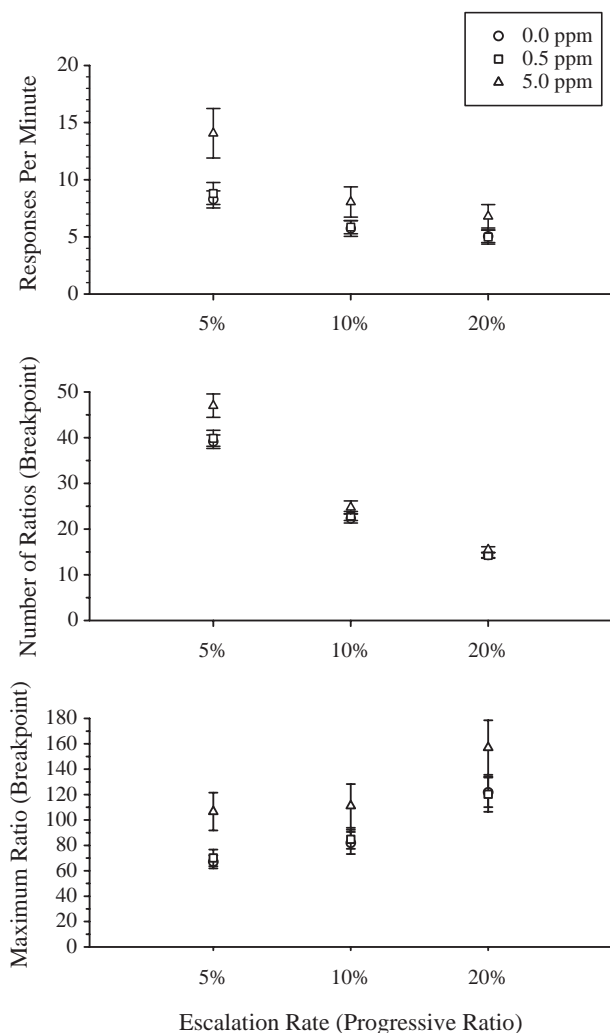


Fig. 5. Responses per minute (top) and breakpoints—number of ratios completed (center) and largest, or maximum, ratio completed (bottom) for the PR experiment. Shows data for each MeHg group arranged according to smallest-to-largest escalation rate. Error bars represent 1 SEM.

comparisons for the 5% rate revealed that the 5 ppm group produced significantly higher response rates than the 0 ppm group ($P=0.01$), but there was no difference between the 0.5 and 0 ppm groups ($P=0.8$); the 5 and 0.5 ppm groups were also significantly different ($P=0.02$).

3.4.2. Breakpoints

There was a significant interaction between MeHg and escalation rate for the breakpoint measure, number of ratios [$F(4,80)=4.5$, $P=0.01$]. However, there was no statistical interaction between MeHg and escalation rate for the maximum ratio breakpoint measure [$F(4,80)=0.3$, $P=0.8$] or any of the secondary breakpoint measures [all F 's(4,80) <0.6 , P 's >0.6]. For every breakpoint measure, there was no interaction among MeHg, Diet and escalation rate [all F 's(4,80) <1.8 , P 's >0.1] nor between Diet and escalation rate [all F 's(2,80) <0.9 , P 's >0.4].

Fig. 5 shows breakpoint data for the number of ratios completed (center) and maximum ratio (bottom) for the three escalation rate conditions. For number of ratios completed,

differences appeared for the 5% session, with the 5 ppm rats completing more ratios, on average, than the 0 or 0.5 ppm rats. Contrasts confirmed this observation. Only the 5% rate produced a significant difference ($P=0.01$), and the 5 ppm group completed more ratios than the 0 ppm ($P=0.01$), although there was no difference between the 0.5 and 0 ppm groups ($P=0.8$); the 5 and 0.5 ppm groups were also significantly different ($P=0.02$). For maximum ratio (bottom), the 5 ppm group averaged larger ratios than the other groups during the 5% session. As reported above, there was no statistical interaction between MeHg and escalation rate for maximum ratio, precluding a need to perform contrasts. However, in light of the apparent, graphical difference between the 5 and 0 ppm (and 5 and 0.5 ppm) groups for maximum ratio, especially during the 5% escalation rate, contrasts were performed. Despite the lack of statistical effect for the omnibus test, the contrasts did confirm graphical observations, revealing a significantly higher maximum ratio for the 5 ppm rats than for the 0 ppm rats ($P=0.01$) during the 5% condition, but 0.5 and 0 ppm rats did not differ on this measure ($P=0.8$); the 5 and 0.5 ppm groups were also significantly different ($P=0.02$). No such findings were observed for any of the secondary breakpoint measures (graphical results not presented).

3.4.3. Cumulative records

Fig. 6 compares within-session data from a 0 ppm rat (top) and a 5 ppm rat (bottom) across time for the 5% session. The 0 ppm rat responded at low, gradually reducing rates culminating in an extended breakdown toward the end of the session (after 115 min). In contrast, the 5 ppm rat responded at a high, steady rate for roughly 23 min before its behavior began to break down, but its high, steady rate recovered on two separate occasions, at about 50 min and again at 103 min into the session. Although the cumulative records for the rats within these two groups varied, these records typify those of several rats within respective groups.

3.5. Quality control

Except for the first session of the FR 1, there was never a significant main effect of squad on response rate. By the third session, and for all remaining sessions of the three experimental phases, there were no differences among squads [all F 's(2,43) <1.9 , P 's >0.1].

Response rates were not affected each day by the chambers in which the animals were performing [all F 's(15,30) <1.6 , P 's >0.1].

Comparisons of 9- and 12-month-old rats revealed that their response rates did not differ within either the CO/0-ppm exposure group [$F(1,6)=1.5$, $P=0.3$] or the CO/5-ppm exposure group [$F(1,6)=0.7$, $P=0.4$], or when both of these groups were combined [$F(1,14)=2.8$, $P=0.1$].

4. Discussion

The behavioral effects of lifetime's consumption of a diet rich in *n*-3 PUFAs and gestational exposure to 0, 0.5, or 5 ppm

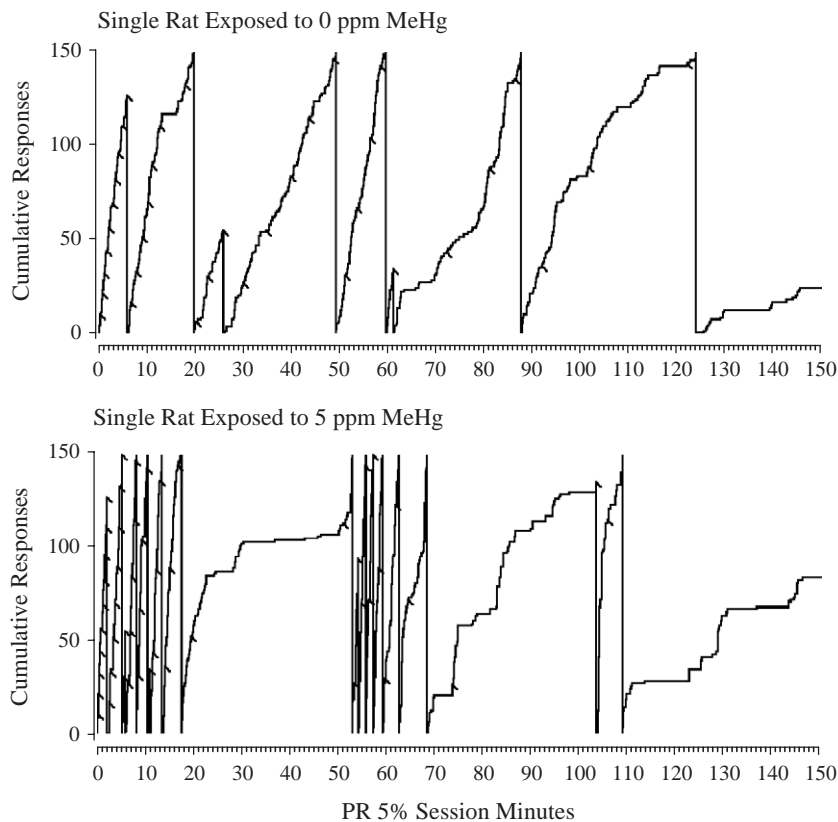


Fig. 6. Cumulative records for two rats from the 150-min session of the PR 5% condition. The top record shows data from a control MeHg rat; the bottom record shows data from a rat exposed to the high dose of MeHg. Steep slopes indicate faster responding. Plateaus indicate periods with no responding. Hash marks indicate times when reinforcers were delivered.

of MeHg were assessed during adulthood. To accomplish this, a 2 (Diet) \times 3 (MeHg) factorial experiment was employed, permitting the examination of main effects of both MeHg and Diet, as well as their potential interaction. Prenatal exposure to 5 ppm, producing about 400 $\mu\text{g}/\text{kg}/\text{day}$ of MeHg, resulted in elevated response rates during transitions to a large fixed ratio schedule (FR 25 and FR 75), in the initial session of a transition to a DRL schedule, and transitions under a progressive ratio when the response requirement increased by a rate of 5% with each reinforcer. The high dose of MeHg also weakened response efficiency during the first session of the DRL schedule, and increased breakpoint number of ratios and maximum ratio during the PR 5% session.

It has been hypothesized that *n*-3 PUFAs in fish oil may protect against MeHg exposure effects. There was no indication here of an interaction between Diet and MeHg. Moreover, no effects were observed involving Diet alone either. The experimental procedure was sensitive enough to detect effects of MeHg, so it should have been sensitive enough to detect similarly sized effects involving the diets had they been present.

Methylmercury's effects only became apparent under certain schedule parameters. Small FR schedule requirements, for example, apparently did not sufficiently challenge behavior such that MeHg groups could be distinguished, but large FR schedule requirements did. This is identical to a conclusion reached with a similar procedure (rapidly increasing FR

schedules) but with developmental exposure to cadmium [45]. There, as here, response rate increases in developmentally exposed animals became apparent only with transitions to larger FR parameters while exposure groups were indistinguishable when only one or five responses were required for food reinforcement.

As with other neurotoxicants and drugs, the procedure employed here was effective in isolating effects of MeHg. One practical strength of this procedure is that it allowed effects on high-rate operant behavior to be seen quickly. The FR phase required only 12 sessions, about 2 weeks, before an effect was observed. In the PR phase, an effect was found even within a single session, although only after exposure to the previous phases. Another procedural strength is that responding to a simple ratio schedule, like FR 1, is easy to train relative to responding on some types of operant schedules, reducing the time and effort required to prepare animals for experimental tests of learning.

4.1. Schedule-controlled behavior

The present findings indicate a reproducible effect of MeHg on performance under two high-rate schedules of reinforcement, the FR and PR. One other high-rate schedule has been employed in the MeHg literature—the differential-reinforcement-of-high-rate (DRH) schedule. As with the FR and PR, the DRH requires a certain number of responses, but

unlike the other schedules, there is a time limit on the emission of each set of responses (e.g., 5 responses required within 2 s). These schedules engender high rates of responding for two reasons [74]. First, at a molar level, response rates dictate reinforcement rates; faster responding produces higher reinforcement rates and few responses means few reinforcers. Second, at a more molecular level, under these schedules reinforcers tend to occur during a pattern of high-rate response bursts, thereby reinforcing that pattern of responding.

To date, only two studies of MeHg and behavior under FR schedules have been reported. In one [23], no effects of prenatal exposure to 50–90 µg/kg/day were found with monkeys, but the procedure was somewhat different from that used here. In the other study with adult pigeons [34], a transient effect was reported after chronic, postnatal MeHg exposure to high doses, averaging between 600 and 1500 µg/kg/day. In both studies, steady state, rather than behavior in transition, was reported.

In one early study of prenatal exposure to 5–50 µg/kg/day MeHg by gastric intubation on gestational days 6–9 [5], rats showed impaired acquisition of DRH performance. However, in that study [5], subject, irrespective of litter, was used as the statistical unit of analysis rather than litter itself, limiting the generality of this work (see Ref. [41] for further discussion). In a more recent study, in which litter was the statistical unit and gestational exposure occurred continuously via drinking water [42,51], there was no effect of 0.5–6 ppm of MeHg on acquisition of DRH performance but exposed rats showed dose-related impairments as they aged. In this study, DRH responding by exposed adults also showed increased sensitivity to amphetamine and reduced sensitivity to pentobarbital [51]. Coupled with the present FR and PR effects, these findings indicate that MeHg interferes with high-rate responding, but not in ways indicative of simple motor deficits and in ways that apparently involve catecholamine and perhaps GABA neurotransmitter systems.

The microstructure of responding was assessed in an attempt to determine what components of response rate produced the elevations in FR responding for the rats exposed to the high dose. It was found that this MeHg effect was associated with a change in the entire distribution of IRTs. Because long IRTs consume so much session time, it is likely that the decrease in the 90th percentile from about 5–6 s in the control group to about 2–3 s in the high-dose group was largely responsible for the exposure-related increase in overall response rate. This suggestion is consistent with the report that reduced DRH response rates are more reflective of pauses between bouts of DRH responding than of the microstructure of high-rate response bouts *per se* [42]. The IRT analysis reported here excluded the post-reinforcer pause (PRP), the time between a reinforcer delivery and the next response. PRP percentiles were also analyzed, but the results were not presented above because too few reinforcer deliveries occurred during the FR 75 sessions to make these results meaningful.

MeHg's effect on the distribution of IRTs during the FR could also explain why response rates were elevated for the high-dose group during the initial DRL sessions. For the FR

75, the median IRTs (50% percentile) ranged from about 0.9 s for controls to about 0.5 s for the 5 ppm group, and few rats had IRTs in excess of 10 s (see Fig. 3). During the DRL phase, FR-typical (i.e., high-rate) response bursts would not be reinforced because an IRT had to be longer than 10 s. It took approximately four sessions before the behavior of the high-dose rats became comparable to that of the low-dose and control rats (see Fig. 4). Interestingly, even though the high-dose rats were responding inefficiently at the start, they still collected roughly the same number of reinforcers as the other two groups within each session. Inspection of raw data (not shown) indicated that during the first DRL session, bursts of short IRTs were intermittently separated by longer, often reinforced IRTs similarly across all MeHg groups. What differed for the high-dose group was that the response bursts contained more responses than the other groups with even shorter IRTs between responses, thereby raising overall response rates and reducing efficiency, but without reducing the number of reinforcers per session. By the final DRL session, there were fewer intermittent response bursts for all groups, and the emission of primarily long IRTs led to more paced responding and higher reinforcement rates.

MeHg-exposed rats responded differently during the initial DRL sessions despite the fact that rates were very similar across groups during the final FR 5 condition, which replicated the first FR 5 condition, imposed before transitioning to the DRL schedule. DRL performances for the high-dose group recovered by the final session to levels similar to those of the other two groups, suggesting any influence of the previous experimental manipulation had worn off. It is perhaps best to view the DRL results as perseveration of high-rate responding during the transition to low response rates rather than an effect on DRL responding *per se*, especially since previous research has demonstrated no MeHg effect on DRL responding [19].

The PR phase generally replicated the elevated response rates seen in the FR phase and permitted parametric exploration of the conditions under which this effect might appear. When the ratio increased at a rate of 5% after each reinforcer, 5-ppm exposed rats had higher response rates and progressed to larger ratio values than controls. This tendency was seen under other progressive ratio conditions too.

Multiple definitions of breakpoint have been posited in the literature (for a review, see Ref. [65]), and two that are commonly adopted are number of ratios completed (e.g., [17]) and maximum ratio obtained (e.g., [30]). These definitions reflect how quickly the subject progresses through the escalating ratios, when it stops responding, or both, but it is never clear which unless additional data are provided (e.g., cumulative records). If it is reported that a subject continued to respond the entire session, number of ratios and maximum ratio will reflect how quickly it responded. If, instead, the subject stopped responding midway through the session, then these ratio measures will indicate how far it progressed before stopping.

Different breakpoint measures were assessed to determine if one characterized the data better than another. The number of ratios obtained, but not latency to a long pause (variously

defined), was affected by MeHg exposure when the ratio escalated at the slowest rate (5%). The omnibus result for maximum ratio was not statistically significant, but post-hoc comparisons still revealed a difference between the high-dose group and the other two groups on this measure. The relatively poor sensitivity of the omnibus test may be due to the way that the ratio value increased. The ratios progressed exponentially within a session so the largest ratio obtained was quite different across rats and, accordingly, there was an excessive amount of variability within the group reaching larger ratios, the high-dose group. Logarithmically transforming the maximum ratios did not appreciably reduce this variability (results not reported).

4.2. Potential behavioral mechanisms of MeHg-induced impairments

With FR or PR responding, adverse effects of drugs or neurotoxicants are often manifested as reductions in response rate, reflecting motor deficits or reduced efficacy of the reinforcer (e.g., anorexia) [38,40,49,65]. In the present investigation, however, response rates increased in the methylmercury-exposed rats. This is not the only example of such an apparently paradoxical effect. Similar rate increases have been reported after developmental exposure to cadmium [45] and TCDD [31]. The elevated response rates may reflect MeHg-induced perseveration or, relatedly, diminished sensitivity of behavior to a change in the reinforcement contingency. This outcome could also reflect increased efficacy of the primary reinforcer, here, sucrose.

After sizeable increases in the response requirement, the exposed rats persisted in lever-pressing, indicative of perseveration, whereas the behavior of unexposed rats decreased and became erratic, a pattern suggestive of extinction. These effects are consistent with diminished sensitivity to differences in reinforcement contingencies, a mechanism that has been suggested in previous studies involving prenatal exposure to MeHg and transitional behavior [46,47]. Thus, the high-dose rats continued responding at high rates under the FR 75 or at large and progressively increasing ratios, suggesting that their behavior was insensitive to this decrease in the reinforcer rate.

A motivational account may also be applied here. Fixed and progressive ratio schedules are used in other contexts to measure the reinforcing efficacy of drugs or other reinforcers [3,29,65]. As the ratio increases it eventually reaches one so large that responding breaks down; this is called the breakpoint and is widely viewed as a measure of the efficacy of reinforcing consequences [58]. This phenomenon is evident in the cumulative records, which showed erratic responding at higher ratios in the controls, but persistent responding in the exposed animals. Applying such an interpretation in the present study would suggest that MeHg exposure increased the reinforcing efficacy of the sucrose pellets because they supported more responding as the ratio increased.

This motivational interpretation of MeHg's effects is consistent with the observation that long interresponse times, pauses, were shorter for the MeHg-exposed rats. Pausing under

ratio schedules of reinforcement, either in steady-state or in transitions to larger schedules, is associated with the same parameters that influence reinforcer efficacy [35,61]. Pausing is shorter if the upcoming conditions are relatively favorable, if the reinforcer is highly preferred, or under conditions of increased deprivation [1,50,67]. Alternatively, pauses are longer if an alternative activity or a more preferred reinforcer is available [18,58], if there is a rapid increase in the response requirement [1], or under conditions of satiation [58,61].

The question remains as to why, with the PR schedule, the MeHg effects appeared only when the ratio increased at 5% per reinforcer, and not at higher rates of increase. Response requirements only increase under a progressive ratio schedule, and it has been shown that pause durations increase and response rates decrease exponentially as the ratio requirement becomes more demanding [1]. It is plausible that under the PR 10% and PR 20% conditions, responding, irrespective of exposure group, became so disrupted so quickly that it was difficult to distinguish the effects of MeHg.

In light of the above discussion, it would appear that higher response rates and shorter pause times seen in the rats exposed gestationally to MeHg could reflect any of several mechanisms: diminished sensitivity to a change in the reinforcement contingencies and consequent perseveration, enhanced efficacy of the primary reinforcer, or diminished reinforcer efficacy of other available activities. Further research will be required to tease these mechanisms apart, but it is worth noting that they are not incompatible or mutually exclusive explanations. In one account, MeHg-exposed rats' behavior could be viewed as less sensitive to the increasing reinforcement contingencies. The other would note that less time was allocated to other, perhaps less reinforcing, activities such as exploring, scratching, and rearing. These possibilities would each result in a response pattern of persistent lever-pressing without interruption, as seen here. This suggestion is also consistent with the slower acquisition of DRL schedule responding and with previously reported observations that prenatal MeHg exposure diminishes sensitivity to reinforcement in procedures that examine choice in transition [46,47].

4.3. Diet considerations

Diets are complex mixtures and several factors must be considered when designing an experimental intervention to examine the role played by a single component such as DHA, or a single class such as *n*-3 PUFAs. These factors include (a) the presence of other *n*-3 PUFAs, (b) the concentration of *n*-6 PUFAs in the two diets, (c) the ratio between *n*-6 and *n*-3 PUFAs, (d) the concentration of saturated fats and (e) the number of generations of depletion. In the present investigation, the fish oil diet contained DHA but the coconut oil diet did not. Both diets did contain ALA, another *n*-3 PUFA that rapidly undergoes biosynthesis to DHA. Therefore, despite having no EPA or DHA, the CO diet contained a DHA precursor, ALA. The conversion rate of ALA to DHA is particularly low [22], however, so the concentration of DHA in the brain should be much lower for rats consuming the CO diet.

Analysis of the fatty acid profile of neonatal (PND 1) brains from siblings of the rats studied here confirmed that the DHA content in the brains of the CO rats was lower than that of the FO rats [60].

Another consideration is the *n*-6 to *n*-3 PUFA ratio, which is thought to be ideal in the range of 2–4 [68,69,73]. The diets for the present study were designed to have *n*-6 to *n*-3 ratios of 16.5:1 (FO) and 2:1 (CO) while holding the *n*-6 concentration relatively constant between the two diets. These ratios were designed to approximate diets that should produce either high or marginal levels of DHA in the brain; i.e., not to be so excessively rich or deficient in DHA that it might have readily produced effects of Diet alone, as has been attempted elsewhere [9,28].

Brain concentration of DHA is tightly regulated and vigorously defended, so shortages of DHA, and its precursors, in the diet may not have an immediate impact. It may take several generations of *n*-3 deficiency in rats receiving a CO-like diet before DHA depletion begins to appear in the adult brain [2]. Here, the dietary deficiency was in the first generation offspring, so it is reasonable to suspect that DHA depletion was relatively marginal, as intended, although fatty acid profiles for the adult brains have yet to be determined. If dietary deficiencies across subsequent generations were to occur, the CO diet might begin to contribute to behavioral effects, possibly in combination with MeHg. However, such a situation would represent neither physiologically relevant concentrations of nutrients nor exposure regimens likely to be experienced by people and, therefore, would be irrelevant to practical considerations of interactions between MeHg and PUFAs.

Saturated fats differed between the two diet groups. Although the two diets were designed to hold *n*-6 concentrations constant while establishing concentrations of *n*-3, and ratios of *n*-6 to *n*-3, that were physiologically relevant, this was accomplished by replacing the *n*-3 (DHA and EPA) in the FO diet with saturated fat (in coconut oil) in the CO diet. It is not possible with such a mixture to hold everything constant. Thus, the experiment could also be viewed as a comparison between saturated fats and *n*-3 PUFAs.

4.4. Concluding remarks

The objective of the present investigation was to isolate exposure to MeHg and fish oil rich in EPA and DHA, and to determine if including or excluding the fish oil or the saturated coconut oil in the diet diminishes MeHg's effects on behavior. Here, neither outcome was observed with the two diets. What was observed was that gestational MeHg exposure altered learning, and specifically the transitioning of high-rate behavior in rats, consistent with previous reports employing similar exposure regimens [42,47].

The PR phase was undertaken in an effort to replicate the effects seen in the FR phase and to examine the role played by speed with which response requirements increase. Use of PR schedules also permitted an examination of reinforcer efficacy, a procedure widely used for this purpose (see Ref.

[65]). The analysis of the interresponse time distributions was undertaken in order to identify the class of responses, high-rate bursts (short IRTs) or pauses (long IRTs), that contributed to the observed elevation in responding. The additional experiments and analyses also entailed additional statistical comparisons. This approach might be viewed as increasing the risk of a Type I error (i.e., falsely claiming that an effect exists), a concern when conducting a large number of independent, unrelated comparisons in complex behavioral studies [63]. The risk of a Type I error due to conducting multiple comparisons is dwarfed, however, by the information available from such a replication, especially when it contributes to multiple lines of evidence that the effects are real [4]. In fact, these additional analyses, especially the replication, diminish the likelihood that the results of the FR phase are sporadic and thereby elevate confidence in the veracity of those results.

The present study's regimen was designed to mimic human exposures by providing chronic exposure to diets and MeHg, by adjusting diets so as to be within a physiologically relevant range, by initiating dietary exposure before exposure to MeHg and initiating MeHg exposure prior to breeding, by continuing exposure throughout gestation, and by using a range of MeHg doses that are relatively low compared with other rodent studies [6,19,21,24] and that produce brain concentrations identified as low to moderate [7,44].

The average daily intake of MeHg in humans has been estimated at 0.01–0.09 $\mu\text{g}/\text{kg}/\text{day}$ (see Refs. [10,39]), and the current reference dose (RfD) established by the U.S. Environmental Protection Agency is 0.1 $\mu\text{g}/\text{kg}/\text{day}$ [39]. The effects seen in the present investigation were specific to the 5 ppm dose, an exposure of approximately 400 $\mu\text{g}/\text{kg}/\text{day}$. This dose may be adjusted down to 40 $\mu\text{g}/\text{kg}/\text{day}$ to account for differences in the toxicodynamics of MeHg in rat versus primate blood (see Refs. [36,44]), making the Lowest Observed Adverse Effect Level (LOAEL) about 2.5 orders of magnitude above the current RfD.

Acknowledgment

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